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VALORIZATION OF RED RICE WINE LEES AS A PARTIAL WHEAT FLOUR SUBSTITUTE IN SANDWICH BREAD: EFFECTS ON NUTRITIONAL QUALITY AND BIOACTIVE COMPOUNDS

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ABSTRACT

Red rice wine lees (RRWL) were obtained following alcoholic fermentation. The lees were totally de-alcoholized and dried to preserve the nutrients and beneficial components that remained in them. The RRWL powder was then used as a partial replacement for wheat flour (5, 10, 15, 20%, and control sample) in sandwich bread production. The particle size distribution reveals that the smallest particle size (6.54 μm) has the largest dispersion (57.18%), and larger particle sizes exhibit narrower dispersion. A 15% replacement of wheat flour with RRWL flour was identified as the most suitable level among the formulations tested, providing an optimal balance between sandwich bread quality and enhanced nutritional and bioactive properties, with higher protein, lipid, ash, fiber, and bioactive compound contents than the control. Scanning electron microscopy pictures indicated substantial variations in sandwich bread crumb microstructure between the control sample (100% wheat flour) and sandwich bread with 15% wheat flour replaced by RRWL powder. These microscopic changes offer a structural explanation for the deterioration of technological qualities such as reduced specific volume and increased sandwich bread crumb hardness. The valorization of this by-product has contributed to improving the quality of sandwich bread while aligning with the growing demand for foods enriched with bioactive compounds and dietary fiber. At the same time, this approach supports the transition toward a circular economy, a development goal increasingly pursued by countries worldwide.

Keywords: Red rice wine lees, Sandwich bread, Wheat flour substitution, Nutritional enhancement, Bioactive compounds

INTRODUCTION

Colored rice cultivars have recently sparked public interest due to their purported health benefits. Red rice, also known as Dragon Blood Brown Rice (commercial name), distinguishes itself from conventional brown rice by having a reddish-brown grain and a red bran layer. This is a highly nutritious rice that is good for your health and is grown in deep-flooded areas of Vietnam. Red rice is considered as a superfood due to its high nutrient content as well as bioactive compounds with strong antioxidant activity. The main fatty acids are MUFA and PUFA (40.7% and 31%, respectively). γ-tocopherol has a higher concentration (0.67 mg/100 g dry weight) than α-tocopherol. All of these components may have biological benefits for human health in addition to their crumb dietary impacts **(Baptista *et al.*, 2024)**.
Rice wine is an alcoholic beverage made by a fermentation process with starting yeast. It is also regarded as a premium material for wine production, yielding a reddish-brown beverage with a naturally sweet flavor and high nutrient content. When red rice is used to make wine, it contributes new color, phenolic compounds, and a richer/slightly astringent flavor than white rice. However, the color may be kept in the lees (wine lees) after fermentation has finished. Despite the fermentation process, RRWL includes several metabolites and critical elements such as carbs, proteins, fibers, vitamins, minerals, and organic acids **(Kim & In, 2016)**. However, in Vietnam, RRWL are solely used as fertilizer, animal feed, or industrial trash. From traditional wine production, the identification of rice wine lees as a source of nutrients has steadily attracted attention, with studies on their possible use as food additives and fermentation substrates **(Kim *et al.*, 2015; Kwon *et al.*, 2022)**. **Chua *et al.* (2024)** demonstrated that rice wine lees are a by-product rich in protein, sugars, and phenolic compounds, highlighting their potential for valorization in value-added food products. However, utilizing red rice wine lees (RRWL) to convert them into value-added products has not received much attention today. Several previous studies have shown that rice wine lees, herbal wine and fruit wine lees are all considered harmless by-products with many biological applications **(Kim *et al.*, 2015)**. Sake lees have also been shown to reduce lipid accumulation in adipocytes **(Motono *et al.*, 2021)**. In addition, wine lees contain polysaccharides, proteins, lipids, and various phenolic compounds, including flavonoids and anthocyanins, which contribute to their antioxidant properties and health-promoting potential **(Jara-Palacios, 2019; Siller-Sánchez *et al.*, 2024)**. **Caponio *et al.* (2024)** reported that wine lees can be used as a functional ingredient in biscuits, contributing to increased polyphenol and dietary fiber contents in the final product. Rice lees powder has been investigated as a food ingredient, substituting a portion of wheat flour in the making of toffee by **Manaois and Morales (2018)**. Notably, lees from various fermented foods have been found to have prebiotic action **(Hernández-Macias *et al.*, 2021; Li *et al.*, 2019; Zhang *et al.*, 2022)**. *Tapuy* (Philippines rice wine) lees are rich in protein and fiber **(Manaois & Morales, 2018)**. As a result, these intriguing byproducts have the potential to serve as functional foods, particularly prebiotics. As domestic red rice wine production grows annually, so does the amount of RRWL, which might become a low-cost source of minerals and prebiotics. As a result, an efficient method for utilizing the rice wine lees is required.
However, due to its high moisture content, fresh RRWL is susceptible to enzymatic activity, microbial growth, continued fermentation, and quality deterioration during storage. Drying reduces moisture and improves storage stability, handling, grinding, transportation, and uniform incorporation into food products. Although drying may cause some degradation or structural changes in heat-sensitive bioactive compounds, it provides a practical balance between material stability and bioactive retention, while facilitating subsequent processing and food applications. As previously discussed, post-fermentation rice wine lees (RRWL) retain valuable constituents, including proteins, dietary fiber, starch, phenolic compounds, and other bioactive substances. Their incorporation into convenient food formulations may enhance nutritional value and antioxidant capacity.
Sandwich bread is one of the world's most popular foods, known for its ease of preparation and variety. It is also a nutritious, quick-to-prepare breakfast option that provides optimum convenience to consumers. However, there is still a paucity of research on the particle size distribution and morphology of RRWL powder, as well as its application in the processing of beneficial products, such as partially replacing wheat flour in sandwich bread manufacture, as revealed in this work. That is why using RRWL powder to partially substitute wheat flour in sandwich bread recipes has piqued our interest.
From a practical perspective, the research also addresses an application gap by transforming an underutilized byproduct of fermentation into a potentially value-added food ingredient. This approach can contribute to enhancing the value of rice processing by-products and minimizing waste. Leveraging RRWL will bring dual benefits: both enhancing the nutritional value of sandwiches and supporting sustainable food production through adding value to by-products and applying circular economy principles.

The novelty of this study lies in the systematic evaluation of RRWL as a partial replacement for wheat flour and the determination of the addition ratio that provides the suitable balance between enhancing nutritional/bioactive properties and ensuring acceptable bread quality. Rather than simply demonstrating that an agricultural by-product can be incorporated into bread, the study also provides evidence regarding the technical limitations and potential benefits associated with various substitution rates. The objective of this study is to valorize RRWL, a by-product of the fermentation process, by investigating their incorporation into sandwich bread and determining the appropriate substitution level of wheat flour with RRWL flour. The ultimate goal was to develop a nutritionally enhanced sandwich bread with desirable quality characteristics and practical technological feasibility.

MATERIAL AND METHODS

Sample collection and preparation

Red rice is sourced from a local market in Vietnam and used in the fermentation process. After fermentation is completed, the RRWL is removed. This raw material is collected, de-alcoholicized, and dried at 60°C for 3.5 hours until the moisture reaches about 5-6%. The resulting powder is finely milled, particle size distribution verified, and utilized as a partial substitute for wheat flour in the sandwich bread recipe. The nutritional composition of RRWL powder was analyzed, including moisture, protein, lipid, carbohydrate, ash, and fiber content were 5.42±0.2%, 26.15±0.1%, 2.8±0.05%, 61.18±0.5%, 2.5±0.15%, and 7.08±0.22%, respectively. The protein increases were particularly noticeable in RRWL, with protein increasing from 8.5% to 26.15% following fermentation (with a concurrent study conducted). In addition to the RRWL powder, other ingredients for the bread recipe are purchased from local markets, such as wheat flour (Bakers' Choice flour, Vietnam), margarine (Tuong An, Vietnam), fresh milk (TH fresh milk, Vietnam), instant dry yeast (Mauripan dry yeast, Vietnam), sugar, salt, and eggs.

Sandwich bread preparation

Some ingredients in the sandwich recipe have predetermined weights, such as margarine (23.4g), sugar (27g), salt (2.7g), fresh milk (117g), instant dry yeast (1.8g), and egg (54g). The total weight of the recipe is 486.9 grams. The wheat flour used is 261g (for the control recipe R0). The quantities of wheat flour and RRWL powder were calculated using four different formulas: R5, R10, R15, and R20, each of which substituted RRWL powder for wheat flour at a rate of 5%, 10%, 15%, and 20%. The automatic bread machine (PETRUS PE9600, China) was set to bake at 165ºC. It took approximately three hours and twenty minutes to mix, proof, and bake. The moisture content, loaf expansion volume, hardness, bioactive compounds, color, and nutritional composition of the sandwich bread were all tested.

Determination of physicochemical properties and total calories

Total anthocyanin content (TAC): the pH differential method was used to determine the anthocyanin content. Given that the anthocyanin chromophore's structure changed between pH 1.0 and 4.5, the approach was appropriate for determining the total anthocyanin content **(Inácio *et al.*, 2013)**. Buffers with pH values of 1.0 and 4.5 were tested for absorbance at 520 and 700 nm using a UV–VIS spectrophotometer (UV1200, YOKE, China). The calculating procedure was carried out in accordance with the description of **Maran *et al.* (2015)**.
Total polyphenol content (TPC): the Folin–Ciocalteu test method described by **Fu *et al.* (2011)** was used to analyze the TPC. A UV-VIS spectrophotometer was used to measure the absorbance at 750 nm. A suitable blank was used to subtract the background. Using the resulting standard curve, TPC was then computed as mg gallic acid equivalent per 100 grams of sample.
Proximate composition: the moisture, protein, lipid, carbohydrate, ash, and fiber content were determined according to the Association of Official Agricultural Chemists method **(AOAC, 2005)**.
Total calories: the sandwich bread's total calorie content was determined using the approach of **Thompson and Manore (2017)**.
Color measurement: a colorimeter (ColorFlex L2, HunterLab, USA) was used to read the color values (L*, a*, b*) of RRWL powder and bread. L* indicates lightness (0 = black and 100 = white), while a* and b* indicate chromaticity (-a*: greenness and +a*: redness; -b*: blueness and +b*: yellowness).
Bread hardness: the TA.XTplusC-Texture Analyzer (Stable Micro System, England) was used to measure the hardness of bread. The bread loaf was put on the texture analyzer platform after being cut into slices that were 2 cm thick. A cylindrical probe was used to compress the sliced bread with 50% strain. The bread's thickness and diameter were used to calculate the spread factor **(Thuy *et al.*, 2023)**.
Loaf volume expansion (LEV): the volume difference between the dough before proofing and the volume of the bread after baking was used to compute the loaf volume expansion **(Pratama *et al.*, 2024)**.

Scanning electron microscopy (SEM)

A Scanning Electron Microscope (JCM-7000, JEOL NEOSCOPE, Japan) was employed to examine the cooked and dried red rice sample and RRWL powder's microstructure.

Particle size distribution

The average size and size distributions of RRWL powder were estimated by a Laser Diffraction analyzer (Microtrac S3500, Germany).

Statistical analysis

All experiments were arranged in a completely randomized design with three replicates. Results are reported as the mean of three independent replicates (loaves). Data are expressed as mean±standard deviation (STD). Statistical analysis was performed using Statgraphics Centurion XV.I software (USA). Analysis of variance (ANOVA) was used to statistically analyze the experimental data, and the Tukey test was employed to compare differences between treatment means at a 95% confidence level.

RESULTS AND DISCUSSION

Particle size distribution

The particle size distribution of a material is an important analytical characteristic in quality control and research applications because it influences a variety of product qualities. Particle size distribution influences a variety of material qualities, including flowability and transportability (for bulk materials), reactivity, abrasiveness, solubility, extractability, flavor, compressibility, and so on. Foods are often processed and marketed as fine particles. The density, compressibility, and flowability of food powders are greatly influenced by particle size and distribution **(Barbosa-Cánovas *et al.*, 2012)**. **Figure 1** depicts the particle size distribution of the RRWL powder after grinding and sieving. The RRWL powder is incredibly fine, with very small particle sizes. The image shows that the smallest particle size (6.54 µm) results in the highest distribution (57.18%). Larger particle sizes (15.56 µm, 18.5 µm, and 22 µm) show narrower distributions of 8.50%, 19.76%, and 6.98%, respectively. Small particle size is useful in food processing because it increases surface contact area, which improves the powder's solubility, dispersion, and homogeneity. This also makes it easier to employ appropriate powder to process a wide range of meals that require fineness, consistency, and even functional foods.

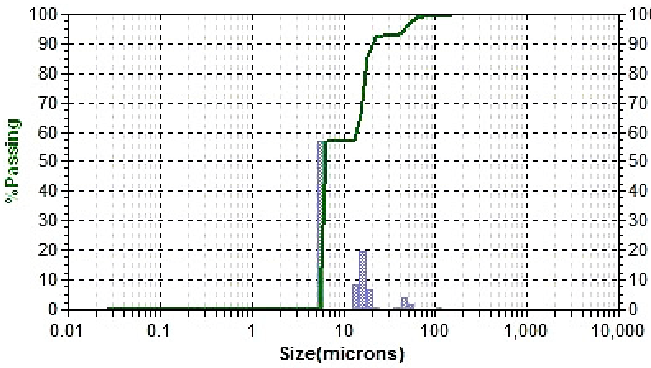


Figure 1 Particle size distribution of RRWL powder

Effect of RRWL powder as a partial replacement of wheat flour in sandwich bread processing

Physicochemical properties of different sandwich bread formulas

Analysis of bread's physical properties is critical for determining product quality and optimizing the manufacturing process. Loaf expansion volume, hardness, wetness, elasticity, softness, crumb structure, and crust color are all indicators of how well the kneading, fermentation, and baking procedures worked. This analysis allows researchers and manufacturers to determine the effect of raw materials on product quality. Furthermore, understanding physical qualities aids in improving stability, extending storage duration, and developing new bread recipes that are consistent with current nutritional and health trends. **Table 1** contains analytical data for sandwich bread made using many alternative recipes.

Table 1 Physicochemical properties of sandwich bread according to different RRWL powder substitution formulas

Formulas	Moisture content (%)	LEV (%)	Hardness (g force)	
			Crust	Crumb
R0	35.11±0.46[a]	81.31±1.34[a]	521.67±14.55[e]	80.34±1.99[e]
R5	33.58±0.32[b]	72.98±2.13[b]	630.72±19.07[d]	116.31±4.46[d]
R10	33.47±0.29[b]	69.04±1.02[bc]	754.82±16.76[c]	140.27±6.65[c]
R15	32.71±0.46[c]	66.15±4.33[c]	905.46±11.24[b]	232.09±6.27[b]
R20	31.84±0.35[d]	40.01±3.04[d]	993.50±34.03[a]	254.70±10.96[a]

Formulas	Color parameters					
	L*		a*		b*	
	Crust	Crumb	Crust	Crumb	Crust	Crumb
R0	71.07±2.79[a]	74.59±1.59[a]	10.24±0.59[c]	4.56±0.58[d]	27.22±0.65[a]	22.12±0.65[a]
R5	57.52±1.51[b]	61.53±0.67[b]	12.23±0.24[b]	6.93±0.24[c]	18.57±1.22[b]	13.34±0.84[b]
R10	49.51±1.09[c]	53.20±1.78[c]	12.96±0.47[b]	7.81±0.22[b]	16.91±0.14[c]	10.36±0.96[c]
R15	44.86±0.63[d]	47.19±1.07[d]	14.58±0.46[a]	7.89±0.38[b]	16.35±0.55[c]	8.15±0.26[d]
R20	42.54±0.91[d]	43.07±0.80[e]	15.23±0.49[a]	9.71±0.38[a]	10.92±0.78[d]	6.05±0.72[e]

Values are expressed as mean±STD (n=3). Mean values with different superscripts in the same column differ significantly at $P \leq 0.05$. R0 represents the control sample, which contains 100% wheat flour. R5, R10, R15, and R20 represent the percentage of wheat flour replaced with RRWL powder (5, 10, 15, and 20%).

Moisture content

The moisture level of bread samples using RRWL powder substitution formulations ranged from 31.84% to 33.58% (**Table 1**). The moisture content of samples R5 and R10, which represent 5% and 10% substitution of RRWL powder, is identical. Among the intended bread samples, sample R20 (RRWL powder replacement 20%) has the lowest moisture content (31.84%), while sample R0 (control) has the greatest (35.11%). The results demonstrate that the moisture content of the bread samples in this investigation is quite comparable to the moisture content range published by **Rammal *et al.* (2024)**, which is between 35 and 40%. Thus, it is obvious that the initial moisture level of the flours utilized in the formulation influenced the difference in moisture content of the finished product. The moisture level of the bread samples reduced when a higher proportion of RRWL powder was replaced, possibly because RRWL powder has a lower inherent moisture content than wheat flour, resulting in a dilution effect as the proportion increased in the recipes.

Loaf expansion volume (LEV)

Sandwich bread's LEV gradually reduced as the RRWL powder ratio increased, and there was a significant difference in LEV between the RRWL powder replacement concentrations and the control sample (**Table 1**). The greatest LEV was recorded in the control sample (R0, 100% wheat flour) and the lowest in sample R20 (20% RRWL powder), which are 81.31% and 40.01%, respectively. This shows that substituting RRWL powder for a portion of the wheat flour will have a negative impact on the bread's LEV. This issue could be caused by a high RRWL powder ratio in the dough, which negatively affects yeast fermentation. On the other hand, adding a high amount of RRWL powder dilutes the gluten protein concentration of the dough, hindering the creation of a continuous gluten network. The reduced gas-holding ability of the dough causes a smaller dough size after fermentation, resulting in a drop in the bread's LEV **(Zanoletti *et al.*, 2017)**. **Tu *et al.* (2025)** discovered that when dried distillers' grains were added at over 6%, the specific volume of the bread dropped. Hence, when the amount of RRWL powder was increased, LEV decreased due to structural alterations in the gluten network of sandwich bread. Dietary fiber can also compete with gluten proteins for water, limiting gluten network development and CO_2 retention, thereby reducing product volume. Studies have shown that fiber-rich by-products can alter the viscosity, rheological properties, and gas-holding capacity of dough systems, consequently affecting bread volume **(Baldán *et al.*, 2023; Bozdogan *et al.*, 2022)**.

Hardness

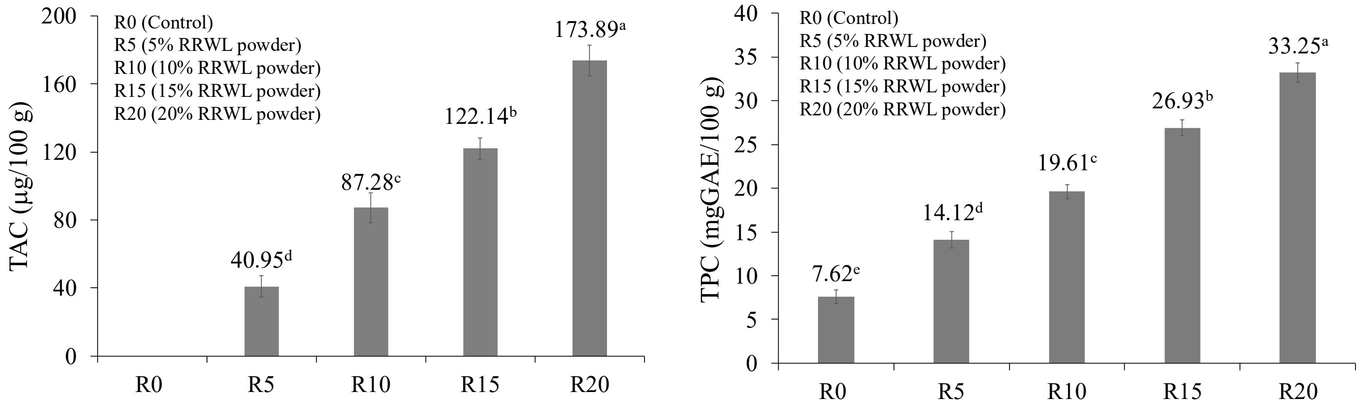
Bread hardness has a considerable impact on taste and is an important consideration for customers when selecting bread **(Gu *et al.*, 2022)**. Bread crust and crumb hardness increase dramatically when RRWL powder content increases (5% to 20%), from 630.72 to 993.50 g force and 116.31 to 254.70 g force, respectively (**Table 1**). This increase could be attributed to the fact that the presence of RRWL powder without gluten protein weakens the gluten network of bread, resulting in poor gas retention. Besides, the addition of fibrous components from RRWL powder dilutes the strength of the gluten protein network and prevents the formation of a three-dimensional network structure **(Chen *et al.*, 2019; Tolve *et al.*, 2021)**, reducing the bread's porosity and increasing its hardness. Likewise, the addition of RRWL powder increases the bioactive components (anthocyanins, polyphenols), which limit the activity of amylase enzymes in the dough, resulting in a lack of maltose for yeast activity during proofing, reducing gas production, hardening the gas cells, and producing bread with a harder bread texture **(Mildner-Szkudlarz *et al.*, 2011)**. This conclusion is consistent with the findings of **Roth *et al.* (2016)** and **Kaur *et al.* (2023)**, who found that adding more dried distillers' grains and *Kinnow* peel increased the bread's hardness. The increase in crumb hardness with increasing RRWL powder content has practical implications for bread formulation. Since crumb hardness is a key textural attribute related to bread firmness and softness perception during eating (Angioloni and Collar, 2009), excessive hardness may result in a firmer, less soft crumb. Therefore, RRWL powder content should be carefully considered during formulation.

Color

Table 1 displays the results of color measurements on the bread crust and crumb according to different RRWL powder substitution formulas. Increasing RRWL powder replacement resulted in lower L* and b* values in the crust and bread crumb, while increasing a* value. This shows a gradual change in the bread's color from golden yellow to brown. Compared to the control bread, both the crust and the bread crumb containing RRWL powder changed color dramatically. This difference could be attributed to the naturally deeper color of RRWL powder. As the RRWL powder content rose, the color of the sandwich bread darkened. Bread crust color depends on the extent of Maillard and caramelization reactions, which are influenced by factors such as moisture, pH, and temperature, producing the golden-brown color of the crust **(Palermo *et al.*, 2012)**. Therefore, the observed color changes may be attributed to the water-holding capacity of RRWL fiber, which alters moisture levels and Maillard reaction conditions during baking. The bacteria that ferment rice wine also produce enzymes like α-amylase and glucosidase. The breakdown of these enzymes produced a considerable amount of reducing sugars, some of which remained in the RRWL granules, implying that this substrate was also responsible for increasing the Maillard process during baking and darkening the bread **(Sayed Ahmad *et al.*, 2018)**.

Bioactive compounds

Figure 2 displays bioactive compounds (TAC and TPC) in sandwich bread made with varying amounts of RRWL powder substituted for some wheat flour. The addition of a range of anthocyanins and polyphenols in RRWL powder is what causes the rise in bioactive ingredients in bread. Prior research has also demonstrated that adding processed by-products of fruits, vegetables, or cereals to bread increases the concentration of bioactive components in baked goods **(Galanakis, 2022; Gómez & Martinez, 2018; Yaqoob *et al.*, 2021)**. The findings in **Figure 2a** demonstrate that TAC was not detected at control sample R0 and that it tended to rise when RRWL powder was used to partially substitute wheat flour from 5% (R5) to 20% (R20) of RRWL powder, with values ranging from 40.95 to 173.89 μg/100 g, respectively. The results showed that using RRWL powder as a partial replacement for wheat flour in bread recipes considerably raised the TAC of the product. RRWL powder naturally contains a high concentration of anthocyanins. On the one hand, they give the product a more appealing, distinctive color; on the other, they increase the functional value of bread (high biological activity and antioxidant activity) and provide health benefits to humans when consumed **(Croitoru *et al.*, 2018; Sompong *et al.*, 2011; Zhu & Sun, 2019)**.



a. b.

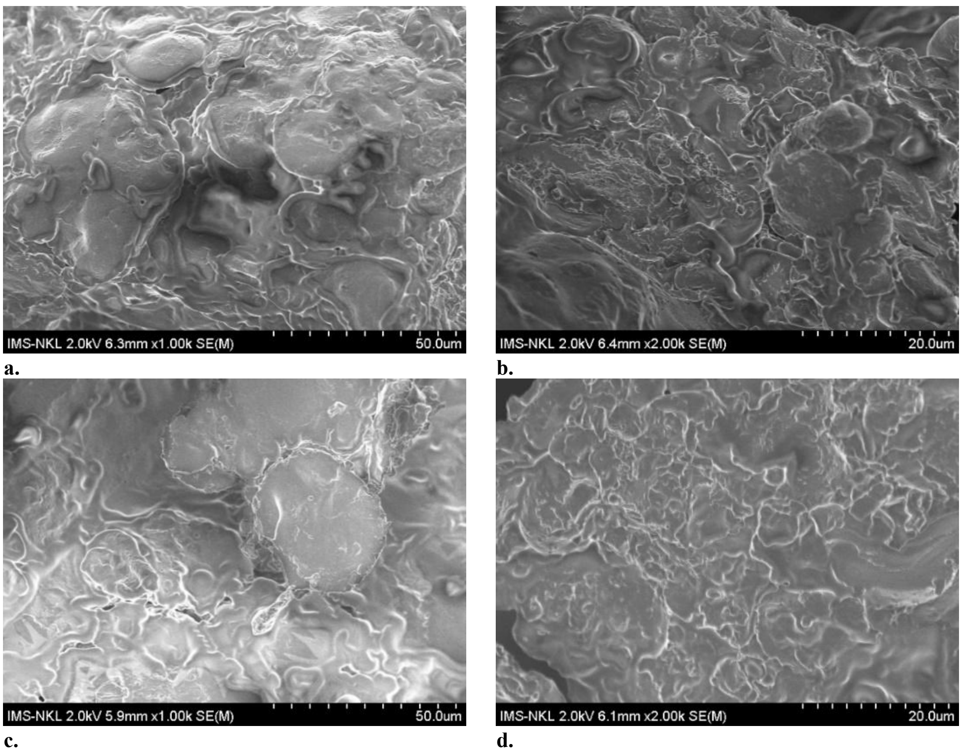
Figure 2. Bioactive compounds [TAC (**a**) & TPC (**b**)] in sandwich bread with different proportions of RRWL powder replacing part of wheat flour

Values are expressed as mean±STD (n=3). Bars with different letters indicate significant differences (P < 0.05)

Phenolic compounds are recognized for their antioxidant capabilities and potential health benefits. In our investigation, using RRWL powder to partially substitute wheat flour enhanced the TPC of sandwich bread considerably. The TPC of various sandwich bread samples rose with the amount of RRWL powder included in the formulations, ranging from 7.62 to 33.25 mgGAE/100 g (**Figure 2b**). Other research found that adding banana peel and mushroom powder to bread boosted the amount of polyphenols **(Akhter *et al.*, 2024; Lu *et al.*, 2021)**. In keeping with this trend, using RRWL powder to partially replace wheat flour in sandwich bread not only makes use of the bioactive compound-rich byproduct, but it can also improve the antioxidant characteristics of sandwich bread and assist consumers looking for polyphenol-rich meals. Although higher RRWL substitution levels enhanced the nutritional and bioactive characteristics of the bread, these benefits were accompanied by more pronounced changes in its technological properties. Therefore, the 15% substitution level provided the most favorable balance between nutritional enrichment and sandwich bread quality among the formulations investigated.

Microstructure of sandwich bread crumb

The microstructure of the sandwich bread crumb seen by SEM (**Figure 3**) indicates a distinct difference between the control sample (100% wheat flour; **Figure 3a-b**) and the sample containing 15% wheat flour and RRWL powder (Figure 3c-d). In the control sample, the gluten network formed almost continuously, with a smooth surface and few fractures. The gluten networks encircling the pores resulted in a homogeneous porous structure with approximately balanced pore diameters. This reflects the high gas-holding capability of the pure wheat flour gluten system during fermentation and baking **(Cauvain, 2012)**.



a. b. c. d.

Figure 3. SEM pictures of the wheat flour control sample (**a**, **b**) and the wheat flour sample replaced with 15% RRWL powder (**c**, **d**) at magnifications of ×1000 and ×2000

In contrast, with 15% RRWL powder supplementation, the gluten network structure became less continuous, with more cracks and insoluble particles scattered in the gluten-starch matrix. The gas cell walls were thicker but rougher and less smooth, with significant shrinkage and collapse seen in some pores. This could be because the gluten content was diminished after partially replacing wheat flour with RRWL powder (rich in fiber), disrupting gluten network formation and lowering the dough's gas-holding capacity. Furthermore, the stability of the dough network is heavily reliant on the secondary structure of gluten protein. Replace it with high-fiber flour, which competes with gluten protein for water. According to **Zhou *et al.* (2021)** and **Xin *et al.* (2022)**, the secondary structure of gluten protein molecules weakens over time, making the gluten matrix readily broken down. At ×2000 magnification (b and d), the differences are even more apparent: the control sample has a smooth surface and an equally dispersed gluten protein network, while the 15% RRWL powder sample has micro-cracks, a rough surface, and partially undissolved particles. This also explains why the bread became somewhat tougher and less leavened as the percentage of wheat flour replaced with RRWL increased. Such microscopic changes provide a structural explanation for the observed decrease in technological attributes, such as lower specific volume and increased crumb stiffness, which are frequently reported in composite breads containing agro-industrial by-products.

Increasing the RRWL substitution level generally enhanced dietary fiber and bioactive compounds but also caused greater changes in the technological properties of the bread, partly due to the reduced proportion of wheat flour and gluten-forming proteins, as well as the added fiber and other non-gluten components from RRWL. Therefore, a higher substitution level does not necessarily provide greater overall benefits. The 15% substitution level represented a balance between nutritional and bioactive improvements and the maintenance of acceptable physicochemical and structural properties under the study conditions.

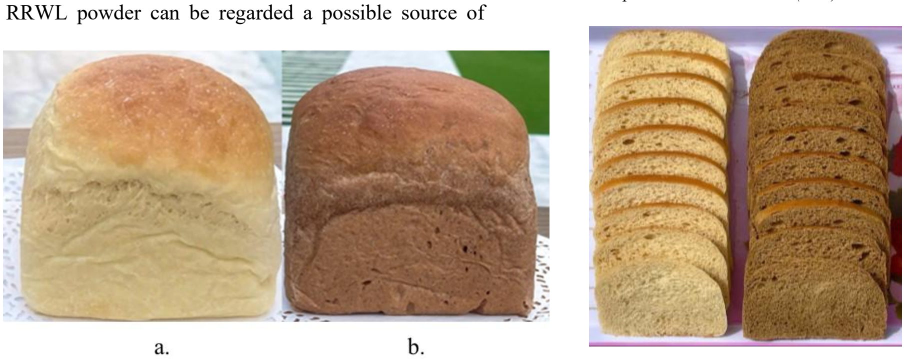
Nutritional data compared between control and 15% RRWL bread recipes

Overall, replacing 15% of wheat flour with RRWL powder in sandwich bread manufacture resulted in significant and positive nutritional benefits. Protein, lipid, ash, and fiber levels all increased significantly in bread samples made with 15% RRWL powder (**Table 2**). This result was owing to the higher concentration of these components in rice lees flour, which increased marginally when partially replaced in the recipe compared to the control sample. However, the moisture content of the bread samples with 15% RRWL powder replacement was lower than that of the control sample, most likely due to red rice flour's poorer water absorption ability than wheat flour **(Islam *et al.*, 2012)**. The carbohydrate content of both samples did not differ much since the contents of wheat flour and RRWL powder were almost equivalent; hence, this content did not change significantly. Thus, it is obvious that RRWL powder can be regarded a possible source of nutrients to increase nutrition without significantly affecting the other key macronutrient components of sandwich breads. **Figure 4** illustrates sandwich bread made using Formulas R0 and R15.

Table 2 Nutritional composition of control sandwich bread and sample with 15% substitution of wheat flour with RRWL powder

Nutritional composition	Control sample (R0, 100% wheat flour)	Selected sample (R15, 15% RRWL powder)
Moisture (%)	35.11±0.46^{a}	32.71±0.29^{b}
Protein (%)	11.8±0.15^{b}	12.9±0.10^{a}
Carbohydrate (%)	48.48±0.3^{a}	48.33±0.23^{a}
Lipid (%)	2.9±0.05^{b}	3.12±0.10^{a}
Ash (%)	1.47±0.08^{b}	1.88±0.05^{a}
Fiber (%)	1.45±0.02^{b}	2.2±0.03^{a}
TAC (µg/100 g)	n.d.	122.14±6.27
TPC (mgGAE/100 g)	7.62±0.75^{b}	26.93±0.91^{a}
Total calories (kcal)	267.22	273

Values are expressed as mean±STD (n=3); "n.d." is not detected.



a. b.

Figure 4 Sandwiches ***a.*** Control sample used 100% wheat flour and ***b.*** Sandwich prepared with 15% RRWL powder in the recipe

Taken together, the results indicate that the 15% RRWL substitution level was selected based on an overall balance among nutritional, physicochemical, and microstructural properties, rather than nutritional composition alone. Although higher substitution levels increased fiber and bioactive compounds, they also caused greater changes in sandwich bread quality due to the reduced proportion of wheat flour and the increased contribution of fiber and other non-gluten components. Thus, 15% was considered an appropriate compromise between improving nutritional and bioactive value and maintaining acceptable physicochemical and structural properties under the conditions of this study. The increased phenolic content, dietary fiber, and antioxidant activity suggest promising nutritional and bioactive potential, although further *in vitro*, *in vivo*, and/or clinical studies are required to confirm potential health benefits.

CONCLUSION

RRWL powder incorporation enhanced the nutritional and bioactive properties of sandwich bread, particularly its dietary fiber and phenolic contents. Particle size distribution analysis provided a physical basis for its incorporation into the bread matrix. Among the tested formulations, the 15% RRWL substitution level provided the most favorable balance between nutritional enhancement and bread quality. At this substitution level, the resulting sandwich bread exhibited improved nutritional characteristics, with protein, fat, ash, and dietary fiber contents of 12.9%, 3.12%, 1.88%, and 2.2%, respectively. The higher phenolic content was associated with enhanced antioxidant activity. SEM analysis further demonstrated that the incorporation of RRWL powder modified the internal microstructure of the sandwich bread. The presence of RRWL resulted in a more heterogeneous sandwich bread matrix, with visible fiber-rich structures distributed throughout the crumb. These microstructural changes provide further evidence of the incorporation and structural contribution of RRWL-derived components within the bread matrix. The valorization of RRWL as a food ingredient offers both environmental and economic benefits by reducing the disposal of an underutilized fermentation by-product and converting it into a value-added ingredient for food production. Rather than being discarded, RRWL can be recovered, processed, and incorporated into new food formulations, such as sandwich bread. Overall, these findings demonstrate the technological feasibility of using RRWL as a partial wheat-flour substitute and highlight its potential for converting an underutilized fermentation by-product into a value-added, nutritionally enriched food ingredient, thereby supporting resource efficiency and sustainable food processing. Further studies should include shelf-life and storage stability assessments, as well as appropriate functional or biological assays to further validate the potential health-related properties associated with RRWL powder incorporation.

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